HISTOLOGY OF THE MANTLE AND PEARL SAC OF THE PEARL OYSTER *PINCTADA MAXIMA* (LAMELLIBRANCHIA)

TREVOR G. DIX *

School of Biological Sciences,

James Cook University of North Queensland, Townsville, Queensland.

Plates 35-36

SUMMARY

The mantle of *Pinctada maxima* is covered by a single layer of epithelium with associated secretory cells. It shows regional differentiation mainly in epithelial cell height, pigmentation, ciliation and nucleus position, and in the distribution and type of secretory cells. Such differentiation relates to variation in the function of different mantle areas, mainly in the secretion of different shell components, and corresponds closely to differentiation found in *Pinctada martensii*.

INTRODUCTION

Extensive studies on the general biology, pearl culture and pearls have been made on the Japanese pearl oyster, *Pinctada martensii* (Dunker). These include investigations of mantle and pearl sac histology and electron microscopy (Aoki, 1966; Ojima, 1952; Nakahara and Machii, 1956; Tsujii, 1960, 1968 a & b). Comparative studies are wanting for the South Seas pearl oyster *Pinctada maxima* (Jameson) a species also of considerable economic importance in pearl culture.

The mantle plays an integral part in pearl culture as a small mantle transplant together with a spherical pearl 'nucleus' are inserted into the visceral tissue of a host pearl oyster during seeding operations. Ideally, the mantle transplant proliferates to form a pearl sac which secretes nacre around the pearl nucleus but in many cases gem quality pearls are not formed. The host pearl oyster may die soon after the transplant operation or, if it survives irregular shaped 'baroque' pearls are often formed. In other animals only organic layer or other low value pearls form.

In view of the frequent occurrence of these undesirable results, investigations of several aspects of pearl culture are being undertaken in *Pinctada maxima.* The present paper forms the basis for investigation of normal mantle processes, the response of the pearl oyster to transplanted mantle and to wounds and the changes occurring in transplanted mantle tissue. It has also proved invaluable for subsequent investigations of histopathological changes accompanying disease.

MATERIAL AND METHODS

For mantle histology eight adult *P. maxima* (dorso-ventral measurement 15.9 - 18.7 cm) were collected in December 1969 from the Old Ground pearling area in the Torres Straits, North Queensland (for locality see Minaur, 1969). A further six pearl oyster collected in this area in August

* Present address: Fisheries Research Laboratory, Crayfish Point, Taroona, Tasmania, 7006.

1968 had had one nucleus and mantle transplant inserted by skilled Japanese technicians in September 1968 and were cultivated until March 1970. Living pearl oysters were airfreighted to Townsville and maintained in a recirculating seawater system for no more than one month prior to dissection and fixation.

After animals were relaxed using propylene phenoxetol (Rosewater, 1963) or magnesium ions (Pantin, 1964) mantle pieces were excised from the following areas and placed is fixative (Fig. 1):

(1) marginal mantle; (a) folds, anterior, posterior and ventral, and (b) isthmus;

(2) pallial mantle immediately inside the fold samples;

(3) central mantle; (a) inside the line of gill attachment and near the adductor muscle; (b) overlying the heart, and (c) overlying the gonad, digestive diverticula and stomach.

Fixatives used were normal Bouins, 10% neutral formalin (Lillie, 1954) and Baker's formol-calcium. In animals containing pearls the visceral mass with pearl *in situ* was removed and placed in fixative. After 20-24 hrs the pearls were removed and the viscera containing pearl sacs were re-immersed in fresh fixative for a further 24 hrs.

Fixed mantle and visceral tissue was routinely dehydrated in alcohol, Paraplast-embedded, sectioned at 5 - 8 μ and stained with Ehrlich's haematoxylin and eosin or Mallory Heidenhain.

RESULTS

MANTLE HISTOLOGY

The gross form of the mantle is relatively unspecialised and consists of two apron-like lobes united dorsally along the mantle isthmus (Fig. 1). Mantle fusion is not present.

Marked regional differentiation, however, was found in the mantle histology and each area will be treated separately.

MARGINAL ZONE

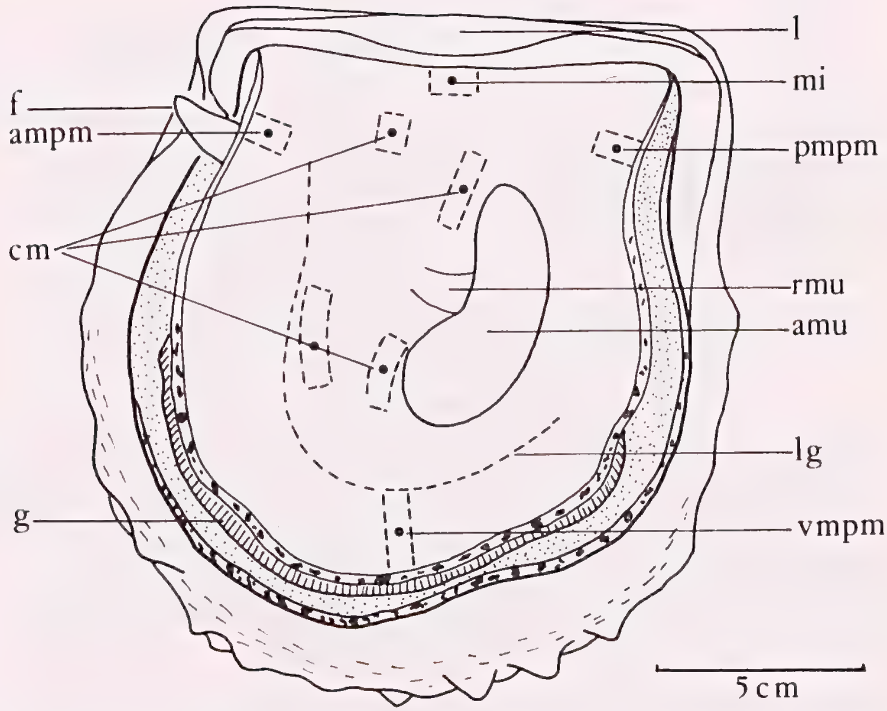
MANTLE FOLDS (Plate 35, Figs. 1-6)

The typical bivalve condition of three marginal folds was found in *Pinctada maxima;* the middle and outer folds are separated by the periostracal groove (Fig. 2). The folds were morphologically similar in anterior, ventral and posterior regions but marked differences occurred between folds.

Inner Fold

A single layer of ciliated columnar epithelium 15 - 20 μ high with basal nuclei covered the largest mantle fold (Pl. 35, Figs. 2 and 3). The distal epithelial cytoplasm was typically pigmented with fine granules which gave a melanin reaction with Lillie's (1957) ferrous ion method and were bleached by the permanganate method (Pearse 1960). At the fold tip ciliation was more conspicuous and pigmentation was reduced.

Conspicuous longitudinal and transverse muscles characterised the inner fold and large numbers of acidophylic secretory cells were scattered through the connective tissue (Pl. 35, Fig. 1). These cells have an amoe-



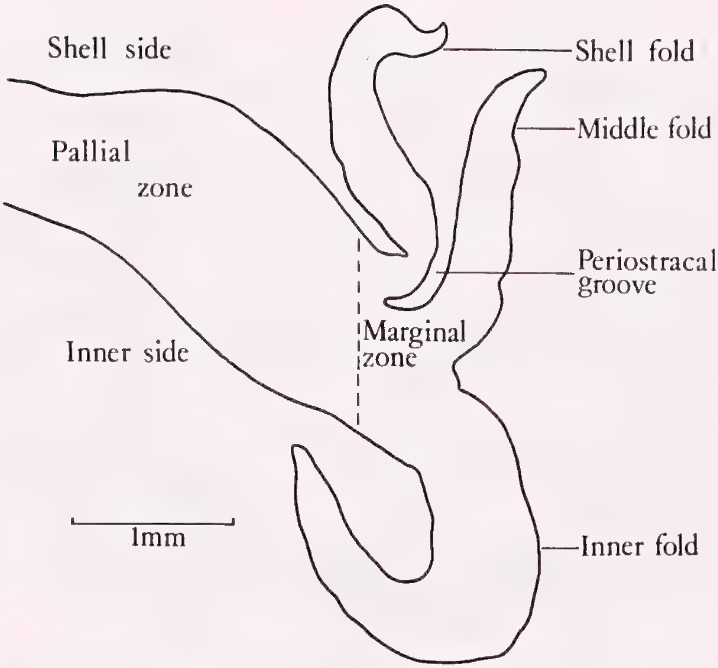
Text Fig. 1: *Pinctada maxima* with the right valve removed to show the mantle regions fixed for investigation. Semidiagrammatic.

boid-like outline, a finely granular cytoplasm and a small but conspicuous nucleus. Although they were most concentrated toward the outer border of the fold they showed little connection with the fold surface. Few secretory cells were found in the epithelium of the inner surface but goblet shaped granular acidophylic cells with large (1 - 5 μ) granules and basophilic mucous cells were common in the outer epithelium and subepithelium, particularly towards the proximal end.

Middle Fold

The middle fold epithelium showed regional differentiation. Cells proximal to the inner fold were similar to those of the latter but the remainder were non-pigmented with lightly basophilic cytoplasm. Non-pigmented epithelial cells of the outer surface were ciliated and similar in size to the pigmented cells (Pl. 35, Fig. 3). A strongly ciliated columnar epithelium reaching a height of 35 μ lined the inner surface of the fold (periostracal groove) apart from near the base which was lined with non-ciliated almost cuboidal cells 8 - 10 μ high, with central muclei and a brush border (Pl. 35, Fig. 4 & 6).

Wandering acidophilic secretory cells which were very abundant in the inner fold were common but less abundant in the middle fold (Pl. 35, Fig. 5). Basophilic mucous cells, however, were extremely abundant



Text Fig. 2: Transverse section through the ventral marginal mantle of *Pinctada maxima* showing the three mantle folds and part of the pallial mantle. Drawn from a histological section.

subepithelially, particularly near the fold tip; they showed clear connections with the fold exterior (Pl. 35, Fig. 3). A few acidophilic cells with large secretory granules were found scattered among the outer epithelial cells although a cluster generally occurred in the subepithelium towards the bottom of the periostracal groove.

Outer fold

The outer (shell) fold showed similar marked regional differentiation to the middle fold but the epithelial cells were neither ciliated nor pigmented.

A clearly demarcated group of tall (40 - 50 μ) stratified columnar epithelial cells occurred at the bottom of the periostracal groove and secreted material on the surface of these cells was continuous with the periostracum in the groove (Pl. 35, Fig. 6). Distally to this group of cells a low columnar epithelium 10 - 15 μ high with lightly basophilic cytoplasm gave way to a taller columnar epithelium (25 - 45 μ high) towards the fold tip. The epithelium of the shell surface of the outer fold was similar although cell height decreased to 10 - 15 μ near the base of the fold.

A group of large lightly basophilic mucous cells occurred below the tall epithelium at the bottom of the periostracal groove. Goblet shaped mucous cells were common along the inner and outer fold surfaces but they were more numerous on the inner surface, particularly towards the tip where acidophilic secretory cells with large granules were also present on the outer fold surface.

MANTLE ISTHMUS (Pl. 35, Fig. 7)

Lying immediately inside the shell hinge line the dorsal marginal mantle or mantle isthmus consisted of a cap of very tall (up to 50 μ) non-ciliated columnar epithelium which rested on connective tissle containing scattered muscle fibres. Several features differentiated the mantle isthmus from other mantle areas. These included strong cytoplasmic basophilia with Ehrlich's haematoxylin, elongation of the cells, and the absence of either epithelial or subepithelial secretory cells.

An abrupt change separated the isthmus cap epithelium from that of the inner isthmus. The latter was similar to that of the central mantle although secretory cells were less numerous near the isthmus.

PALLIAL MANTLE (Pl. 35, Fig. 8; Pl. 36, Fig. 9)

Inner and outer low columnar epithelial layers enclosed muscular connective tissue in the pallial mantle. Although their cells were of similar size (10 - 15 μ x 4 - 6 μ) marked differences occurred between the inner and outer epithelia.

The inner epithelium was characterised by dense cilia, melanin pigment in the cytoplasm and deeply staining basal ovoid muclei. Cilia and cytoplasmic pigmentation were absent in the outer epithelium and the ovoid nuclei were placed basally to centrally with clearly distinguishable chromatin.

Basophilic mucous and coarsely granular acidophilic secretory cells were found on both sides of the pallial mantle but they were small and restricted to the epithelium of the inner surface. The larger secretory cells of the outer surface occurred in the subepithelium as well as the epithelium.

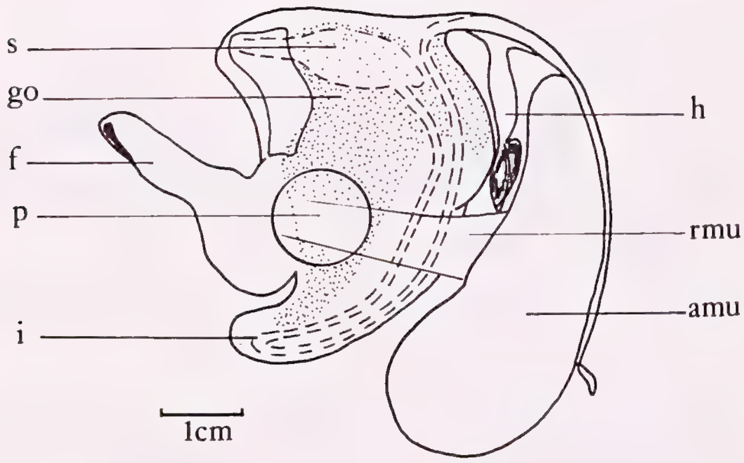
CENTRAL MANTLE (Pl. 36, Fig. 10)

The outer (shell) side of the three sampled central mantle areas consisted of a simple, low columnar epithelium 8 - 10 μ high beneath which and opening through was a characteristic layer of very concentrated secretory cells. The layer consisted of goblet shaped basophilic mucous cells overlying coarsely granular acidophilic cells. The connective tissue containing some muscle cells separated this layer from the gonad and digestive diverticula in the stomach region and from the inner, non-pigmented low columnar epithelium in the heart and adductor muscle regions. Secretory cells in the inner epithelium of the latter central mantle areas were histologically similar to those of the inner pallial mantle.

PEARL SAC HISTOLOGY

All pearls were found in the visceral tissue between the base of the foot and the intestine; they were enclosed by pearl sacs surrounded by gonad, connective and muscular tissue (text Fig. 3).

The following pearl formations were found in the specimens examined: spherical nacreous pearl (2); irregular nacreous pearl without nucleus (4), and irregular periostracal or organic layer pearl without nucleus (1). Prismatic layer pearls were not found in this study.



Text Fig. 3: Viscera of *Pinctada maxima* showing the positon of a spherical pearl. Semidiagrammatic. ABBREVIATIONS: amu — adductor muscle; ampm — anterior marginal and pallial mantle; cm — central mantle; f — foot; g — gill; go — gonad; h — heart; i — intestine; l — ligament; lg — line of gill attachment; mi — mantle isthmus; p — pearl; pmpm — posterior marginal and pallial mantle; rmu — retractor muscle; s — stomach; vmpm — ventral marginal and pallial mantle.

NACREOUS PEARL SAC (Pl. 36, Fig. 11)

Pearl sacs surrounding irregular and spherical nacreous pearls did not differ histologically. They consisted of a single uniform layer of non-ciliated, cuboidal or flattened epithelium 3 - 8 μ high lying in close contact with gonad, connective or muscular tissues. The epithelial cytoplasm was non-granular or very finely granular and slightly basophilic. Nuclei were rounded, centrally placed and occupied much of the cell space.

Two types of secretory cells were found scattered within and immediately below the pearl sac epithelium; these were acidophilic with large secretory granules and less numerous basophilic mucous cells.

PLATE 35

Fig. 1: Inner fold of the marginal mantle of *Pinctada maxima* showing pigmented epithelium (pe), numerous wandering secretory cells (ws) and strong band of muscles (mu). Mallory—Heidenhain x20.

Fig. 2: Pigmented columnar epithelium and secretory cells (sc) of the outer surface of the inner mantle fold in *Pinctada maxima*. Haematoxylin-eosin x325.

Fig. 3: Ciliated, non-pigmented columnar epithelium and subepithelial mucous cells (bs) near the tip of the middle mantle fold of *Pinctada maxima*. Haematoxylin-eosin x325.

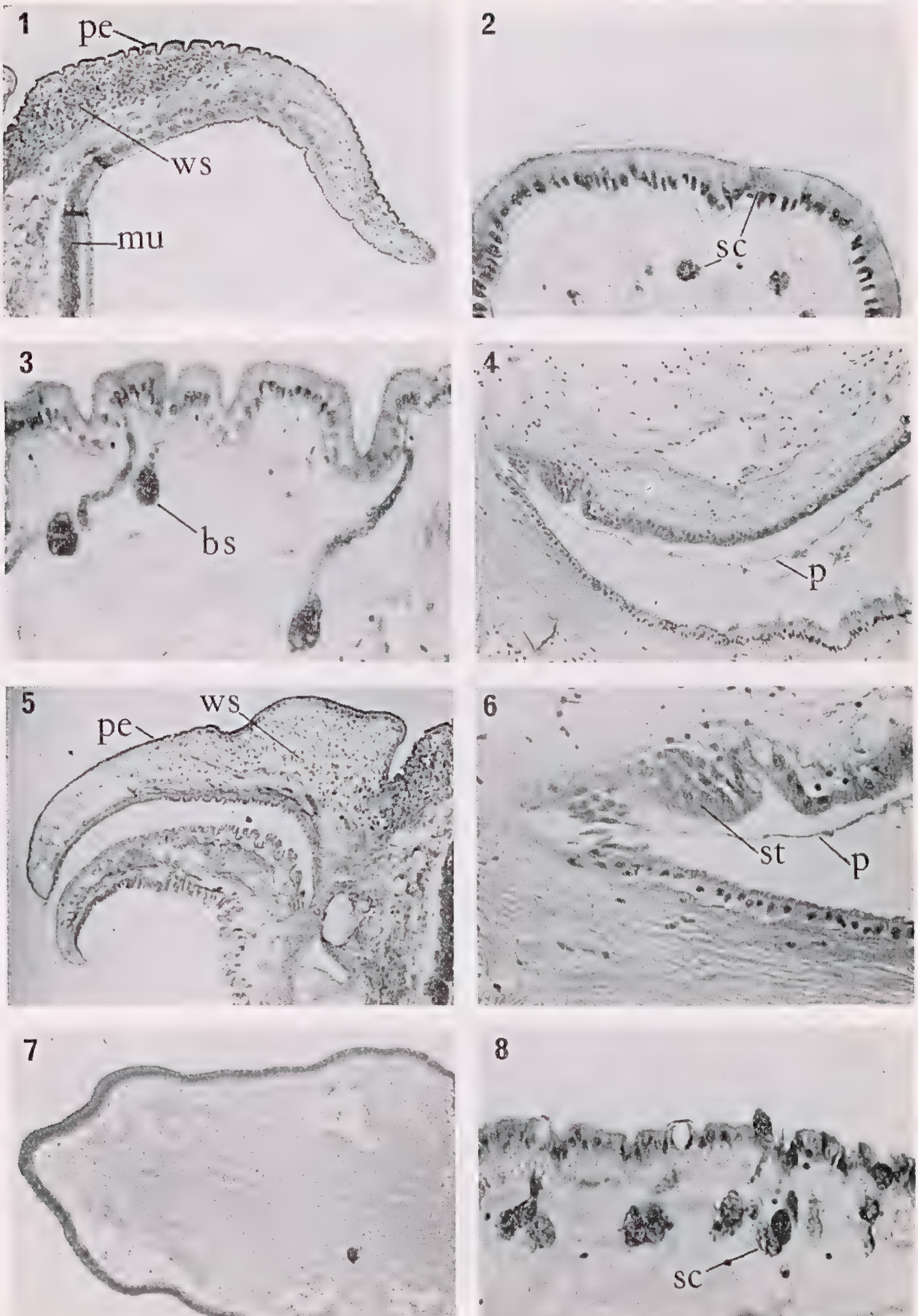
Fig. 4: Epithelia lining the periostracal groove in *Pinctada maxima*. Secreted periostracum (p) is visible in the groove. Outer fold to the top and middle fold to the bottom. Haematoxiyin-eosin x130.

Fig. 5: Middle and outer marginal folds of the mantle of*Pinctada maxima* showing the pigmented epithelium (pe) and wandering secretory cells (ws) of the inner middle fold and non-pigmented epithelium of the outer fold and outer surface of the middle fold. Haematoxylin-eosin x20.

Fig. 6: Stratified columnar epithelium (st) and secreted periostracum (p) at the bottom of the periostracal groove in *Pinctada maxima*. Outer fold to the top. Haematoxylin-eosin x325.

Fig. 7: Strongly basophilic non-ciliated columnar epithelium of the mantle isthmus in *Pinctada maxima*. Haematoxylin-eosin x50.

Fig. 8: Outer, non-ciliated low columnar epithelium and subepithelial secretory cells (sc) of the pallial mantle of *Pinctada maxima*. Haematoxylin-eosin x325.


1
pe
ws
mu
2
sc
3
bs
4
p
5
ws
pe
6
st
p
7
8
sc

PERIOSTRACAL PEARL SAC (Pl. 36, Figs. 12-14)

Most of the periostracal pearl sac constisted of a single layer of tall (30 - 50 μ) ciliated columnar epithelium with basal nuclei and finely granular, slightly acidophilic cytoplasm, but regional differences were evident. Some parts showed a stratified epithelium while others were hyperplastic with extreme proliferation of small (up to 9 x 5 μ) irregularly arranged cells; these cells, which were also concentrated in parts of the pearl sac subepithelium, were probably haemocytes.

Basophilic mucous cells with fine granular inclusions were common in the subepithelium of the pearl sac and acidophilic cells with large secretory granules were present in one marked concentration.

DISCUSSION

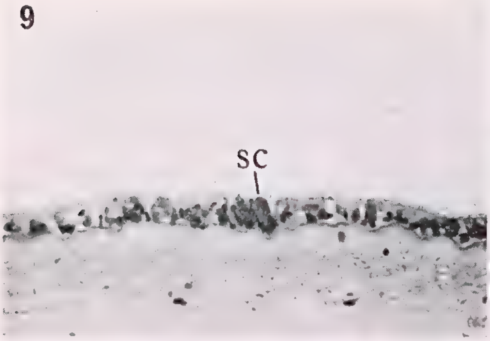
The regional differentiation of histological features found in the mantle of *Pinctada maxima* appears typical for bivalves. Examples include *P. martensii* (Tsujii, 1960), *Anodonta cygnea, Mytilus edulis* and *Ostrea edulis* (Beedham, 1958), *Pinna carnea* (Yonge, 1953), *Crassinella mactracea* (Allen, 1968) and *Mercenaria mercenaria* (Hillman and Shuster, 1962).

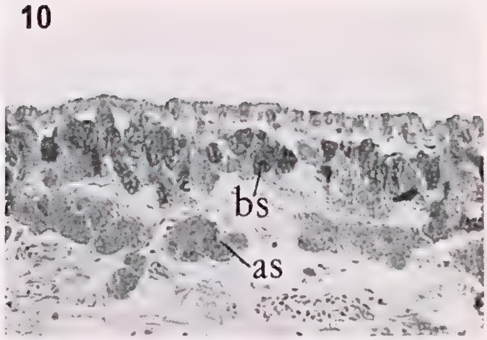
In *P. maxima* the most marked differentiation occurred in the marginal mantle (folds and isthmus). These areas were not only quite different from other parts of the mantle but differentiation was found also between and within the folds.

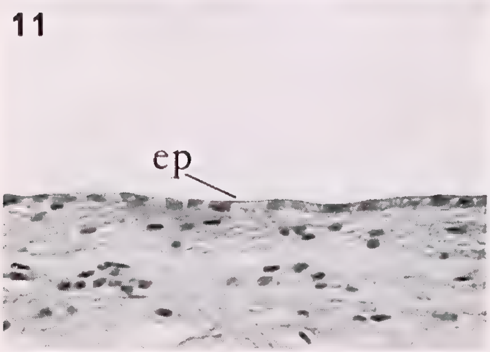
Most of the structural differentiation may be related to function. It is believed generally that the inner, middle and shell folds in bivalves primarily are muscular, sensory and secretory respectively (Beedham 1958; Jorgensen, 1966). This appears true of *Pinctada maxima.* It seems clear that the distinctive tall stratified columnar epithelium on the outer fold at the base of the periostracal groove secretes at least part of the periostracum, particularly in view of the continuity between these cells and newly formed periostracum. A similar group of cells was found in *O. edulis* (Beedham, 1958) while homologous cells extended further along the inner surface of the outer mantle fold in *A. cygnea* and *M. edulis* (Beedham, 1958) and *Mercenaria mercenaria* (Hillman, 1961). Tsujii (1960) suggested that cells of the middle fold secreted periostracum in *P. martensii* although he later (1968a) described a group of cells in the outer fold similar to those found in *P. maxima.*

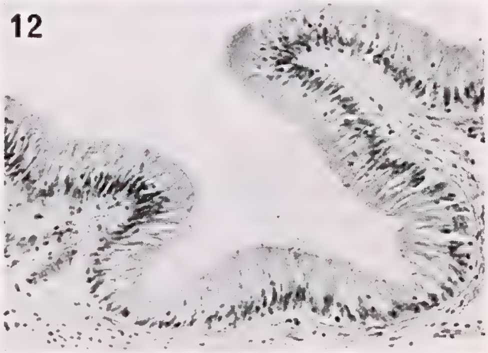
Part of the outer fold probably also secretes the inner calcereous (prismatic) layer of the shell as suggested for *P. martensii* (Tsujii 1968a) and *P. radiata* (Nakahara and Bevelander (1971)). The non-ciliated, non-pigmented columnar epithelium over most of the outer fold differs from that of the other folds and suggests a difference in function.

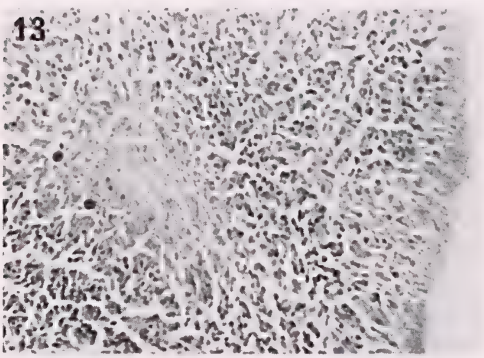
While the inner fold is larger and more muscular than the middle fold both are similarly innervated and may have a sensory function. The inner and middle folds also have ciliated and pigmented epithelia and abundant subepithelial secretory cells further suggesting functional similarity. Conversely, more abundant muscular tissue in the inner fold and more frequent mucous cells in the middle suggest functional differences. The clarification of these must await further study.











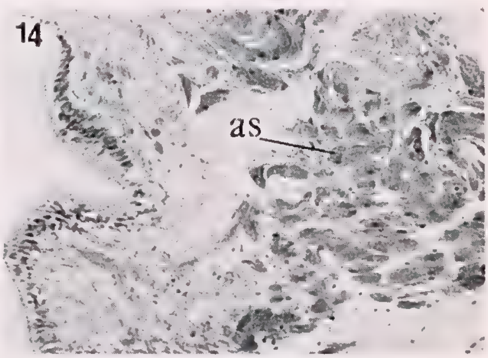


PLATE 36

Fig. 9: Inner, ciliated, pigmented low columnar epithelium and epithelial secretory cells (sc) of the pallial mantle of *Pinctada maxima*. Haematoxylin-eosin x325.

Fig. 10: The outer central mantle of *Pinctada maxima* showing concentrations of mucous cells (bs) and acidophilic granular secretory cells (as) below the low columnar epithelium. Haematoxylin-eosin x210.

Fig. 11: Cuboidal to flattened epithelium (ep) of the nacreous pearl sac in *Pinctada maxima*. The pearl sac is adhered to surrounding connective tissue. Haematoxylin-eosin x500.

Fig. 12: Tall, columnar, ciliated epithelium of a periostracal pearl sac in *Pinctada maxima*. Haematoxylin-eosin x210.

Fig. 13: Proliferated cells (haemocytes) which have replaced part of the periostracal pearl sac epithelium in *Pinctada maxima*. Haematoxylin-eosin x210.

Fig. 14: Concentration of granular acidophilic secretory cells (as) in the subepithelium of the periostracal pearl sac in *Pinctada maxima*. Haematoxylin-eosin x210.

The distinctive columnar epithelium of the dorsal marginal mantle or mantle isthmus *P. maxima* is probably responsible for secretion of at least part of the shell ligament in view of the relationship between shell and mantle in this area. Ligament secretion has been attributed to similar cells as shown by an electron microscope study of *P. radiata* (Bevelander and Nakahara, 1969) and histological studies of *O. edulis* and *M. edulis* (Beedham, 1958).

Because of their relationship to the shell, the outer layers of the central and pallial mantle are likely to contribute to the secretion of the inner calcified layer (nacre). The uniform, low simple epithelium and subepithelial secretory cells in these outer mantle areas differ from corresponding tissues in the marginal and inner mantle areas. The latter, with ciliated epithelium and epithelial secretory cells, may carry out cleansing and rejection of deposited particles as suggested for bivalves in general by Jorgensen (1966).

The marked differences between pearl sacs surrounding nacreous and periostracal materials parallel closely those found in the Japanese pearl oyster *P. martensii* (Aoki, 1966; Nakahara and Machii, 1956; Tsujii, 1960, 1968b). Comparing pearl sacs with mantle areas, the nacreous pearl sac shows general similarity with the outer central mantle although secretory cells are less abundant in the pearl sac. This similarity is expected as both epithelia are concerned with nacre secretion. The periostracal pearl sac shows slight similarity with the tall cells which secrete periostracum at the base of the periostracal groove although these mantle cells are not ciliated. Other differences include the large numbers of haemocytes in the subepithelium and epithelium of the pearl sac and also the extensive folding of the sac. These features may indicate an inflammatory response as suggested for periostracal pearl sacs in *P. martensii* (Aoki, 1966). It is noteable that mantle areas which normally secrete nacre may secrete periostracum under abnormal conditions (e.g. disease or during shell repair). Under these circumstances the mantle shows remarkable similarity to the periostracal pearl sac in the development of elongate cells, cilia and the infiltration of haemocytes (Dix, unpublished observations and Tsujii, 1960).

ACKNOWLEDGEMENTS

The support, facilities and helpful criticism provided by Professor C. Burdon-Jones are gratefully acknowledged. Useful criticism was given also by Dr. C. G. Alexander. Mr. R. Yeldham, Photography Department, James Cook University, printed the photomicrographs.

REFERENCES

ALLEN, J. A., 1968. The functional morphology of *Crassinella mactracea* (Linsley) (Bivalvia: Astartacea). *Proc. Malac. Soc. Lond. 38:* 27-40.

AOKI, Shun, 1966. Comparative histological observations on the pearl-sac tissue forming nacreous prismatic and periostracal pearls. *Bull. Jap. Soc. Sci. Fish. 32* (1): 1-10.

BEEDHAM, G. E., 1958. Observations on the mantle of Lamellibranchia. *Quart. J. Miscroscop. Sci. 99* (2): 181-91.

BEVELANDER, G., and H. NAKAHARA, 1969. An electron microscope study of the formation of the ligament of *Mytilus edulis* and *Pinctada radiata*. *Calc. Tiss. Res. 4* (2): 101-12.

HILLMAN, R. E., 1961. Formation of the periostracum in *Mercenaria mercenaria*. *Science 134:* 1754-55.

HILLMAN, R. E., and C. N. SHUSTER, 1962. Observations on the mantle of the Northern Quahog, *Mercenaria mercenaria* (L.) *Proc. Nat. Shellfish. Assoc. 51:* 15-22.

JORGENSEN, C. B., 1966. Biology of Suspension Feeding. Pergamon Press, London. xv + 357pp.

LILLIE, R. D., 1957. Ferrous ion uptake: a specific reaction of the melanins. *A.M.A. Archives Path. 64:* 100-103.

MINAUR, J., 1969. Experiments on the artificial rearing of the larvae of *Pinctada maxima* (Jameson) (Lamellibranchia). *Aust. J. Mar. Freshwat. Res. 20:* 175-87.

NAKAHARA, H., and G. BEVELANDER, 1971. The formation and growth of the prismatic layer of *Pinctada radiata. Calc. Tiss. Res.* 7(1): 31-45.

NAKAHARA, H., and A. MACHII, 1956. Studies on the histology of the pearl sac. I. Histological observatons of pearl sac tissues which produce normal and abnormal pearls. *Bull. Natl Pearl Res. Lab., 1:* 10-13.

OJIMA, Y., 1952. Histological studies on the mantle of pearl oyster *(Pinctada martensii* Dunker) *Cytologia* 17(2): 134-43.

PANTIN, C. F. A., 1964. Notes on microscopical technique for Zoologists. Cambridge University Press. viii + 77pp.

PEARSE, A. G. E., 1960. Histochemistry. Theoretical and Applied. Edn. 2. Churchill, London. viii + 998pp.

ROSEWATER, J. 1963. An effective anesthetic for giant clams and other mulluscs. *Turtox News 41* (12): 300.

TSUJII, T., 1960. Studies on the mechanism of shell and pearl formation in Mollusca. *J. Fac. Fish., Mie. Pref. Univ.* 5(1): 1-70.

——— 1968a. Studies on the mechanism of shell and pearl formation. X. The submicroscopic structure of the epithelial cells on the mantle of pearl oysters, *Pteria (Pinctada) martensii* (Dunker). *Rep. Fac. Fish. Pref. Univ. Mie 6:* 41-57.

——— 1968b. Studies on the mechanism of shell and pearl formation. XI. The submicroscopic observation on the mechanism of formation of abnormal pearls and abnormal shell. *Rep. Fac. Fish. Pref. Univ. Mie 6:* 59-66.

YONGE, C. M., 1953. Form and habit in *Pinna carnea. Phil. Trans.* (B) *237:* 335-74.